EFFECT OF ADRENALECTOMY ON CALCIFICATION OF RABBIT DENTINE AND ON THE AMOUNT OF SERUM CALCIUM AND TOTAL PROTEIN

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FIVE FIGURES

Since the adrenals are important endocrine glands, their effects on hard tissues like tooth and bone have already been investigated by many, for example, Ikuta4), Yonaga15), Falta2), Schour and Rogoff10), and others71213). Recently, a new vital staining method for hard tissues was originated by Okada and Mimura8). Because the time marked in dentine by this method is evidently visible on its section, the details of development of hard tissues can be grasped exactly in time relation. Using this method, the author has made supplementary studies upon the effect of the adrenals on the development of hard tissues, and observed the changes of calcification and the relation between the growth speed and the amount of serum calcium or total protein much more exactly with regard to time than in any other previous reports.

EXPERIMENTAL METHODS

a) Rabbit is used for the experiment, because its dentine is formed with regular calcification and a stripe figure, which indicates that the qualitative change of calcification can clearly be seen in its dentine.

b) The vital staining injection into vein with 2 mg. per kgm. of lead acetate, which was originated by Okada and Mimura8) in 1938, is applied.

c) The adrenalectomy is performed in the right side at first, and then in the left side eight days later.

d) The tooth together with the maxillary is decalcified in a 1% hydrochloric acid solution into which sulphuretted hydrogen is passed, and then, after freezing, sliced into sections.

e) Observation of qualitative changes in calcification. When the normal dentine of a rabbit incisor is stained with hematoxylin, it shows a calcification figure of stripes regularly stained in light and medium blue, which are respectively formed by day and by night. The less stained part (light blue part) is not calcified well, while the blue part (medially or deeply) is well calcified. In this way, the qualitative change of calcification is observed by the degree of hematoxylin staining on the section. The direction of dentine tubules is traced by staining with thionin-picric acid. And the arrangement of collagenous fibrinogens is observed with the polarizing microscope.

f) Observation of quantitative changes in dentine formation. The rate of dentine formation and growth are determined by measuring the intervals between vital staining lines on the section of the third mandibular molar, cut sagittally in labiolingual direction.

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Schematic figure of periodic stripes

light stained stripe

blue stained stripe

Fig. 1.
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Schematic figure of periodic stripes

unstained stripe  blue stained stripe
light stained stripe  intensively blue stained stripe

Fig. 2.
EXPLANATION OF FIGURES 1 and 2

Fig. 1. Relation among serum calcium, total protein and the periodic stripe of dentine after the incision of the abdominal wall. Serum calcium and total protein, as shown in TABLE 1, decrease immediately after right side incision, reaching the minimum values, 8 and 10% respectively lower than the normal, in the following day, and recover after three or four days. By additional left incision, the calcium value decreases 10% of the normal in the following day, the total protein value also decreases about 10%, and both return after five or six days to the normal level. There is no change in the periodic stripes of dentine. The arrows indicate the vital staining lines (2 mg: per kgm: intravenously injected at 4 days intervals each at 8:30 a.m.) Dentine grew from left to right as indicated by a thick arrow: R.I., right incision of the abdominal wall; L.I., additional left incision of the abdominal wall; P, dental pulp; D, dentine.

Fig. 2. Relation among adrenalectomy, serum calcium, total protein and the periodic stripe of dentine. When the amounts of serum calcium and total protein fall and attain the minimum, in the place of blue and white stripes figure of calcification are formed unstained stripes a) and c). When they rise gradually from the minimum value, a blue stripe and an intensively blue stained stripe d) are formed. R.E. right adrenalectomy; L.E., additional left adrenalectomy; P, dentine pulp; D, dentine.

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<td>b) Ca value rises gradually from 90% to 97%</td>
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g) The amount of serum calcium in venous blood is measured by the Clark-Collips' method. The blood is taken at the definite time in the day.

h) The total amount of protein in the nodemolytic serum from the same blood used in g) is measured with a protein refractometer made by Hitachi Mfg. Co., which measures the refractive index of protein liquid.

The results of e), f), g) and h) are compared with reference to the lead lines by the vital staining to see exactly the time relation.

i) As controls, the amounts of serum calcium and total protein are measured after incision of the abdomital wall without any operation of the adrenals.
RESULTS

a) The amounts of serum calcium and total protein in controls show a slight decrease of about 7 to 15% of the normal, but several days later, they return to the normal (Fig. 1).

No effects are observed on the calcification stripe figure and in the growth rate of dentine (Figs. 1 & 3).

b) Effects of right adrenalectomy. They are observed in eight cases. The calcification of dentine undergoes changes, developing no stripes and presenting a wholly unstained part for one or two days, which is immediately followed by a blue or deep blue stained part for the succeeding two or three days (Fig. 2).

The growth rate of dentine decreases 10 to 20% for the first four days after the operation in five of seven cases, and increases 10 to 25% for the following four days in all cases. The rate of formation tends to change in the same way, though not remarkably (Fig. 4).

On the other hand, serum calcium decreases immediately after the operation, reaches the minimum value which is 5 to 15% lower than the normal in the following day, begins to increase from the second day, and returns to the normal level after seven or eight days without exception. The total amount of protein changes in the same way as the calcium, but decreases more remarkably (Fig. 2).

c) Effect of bilateral adrenalectomy (additional left extirpation). The calcification figure on dentine shows that a unstained part is formed for one to three days after the operation, and a blue or deep blue stained part for the following two to seven days (Fig. 2).

The rates of dentine formation and growth decrease remarkably, being 15 to 29% lower than the normal (Fig. 4).

The amount of serum calcium also decreases remarkably, representing the minimum value 10 to 23% lower than the normal in the following day, and then it begins to increase gradually, but dose not recover the normal value even after ten days. The total amount of protein changes nearly the same way as the calcium, representing the minimum value 20 to 30% lower than the normal in the following day, and then it begins to increase gradually. Its decrease and recovery are more remarkable than the calcium (Fig. 2).

All changes observed after the bilateral adrenalectomy are more remarkable than those after the unilateral.

d) Other changes. The formation of dentikels in the tooth pulp is observed in three of eight cases. No effect on the direction of the dentine tubules and the arrangement of collagenic fibers is observed because of the transverse section.

DISCUSSION

Regarding the effect of adrenalectomy on hard tissues, Schour and Rogoff\(^{10}\), Ikuta\(^6\), Yonaga\(^5\), and others reported that the development of tooth was retarded in quality by adrenalectomy, and the calcification was insufficient in quantity in enamel as well as dentine.
Fig. 3.

Fig. 4.

Fig. 5.
EXPLANATION OF FIGURES 3, 4 and 5

Fig. 3. Relation between the incision of the abdominal wall and the speed of dentine formation. The gilded sample of labiolingually cut section of the 3rd mandibular molar (x 55). Dentine grew from left to right as indicated by a thick arrow. Six white arrows indicate the final points of lead acetate vital staining lines (2 mg. per kgm. intravenously injected at 4 days intervals, each at 8:30 a.m.). A, a part formed in 4 days before the operation; B, a part formed in 4 days after right incision of the abdominal wall; C, a part formed in 4 days after 5~8 days of right incision of the abdominal wall; D, a part formed in 4 days after bilateral incision; E, a part formed in 4 days after 5~8 days of bilateral incision. B, C, D, and E are nearly equal to the preoperative value A. Therefore, the speed of growth is not affected by the incision.

Fig. 4. Relation between adrenalectomy and the speed of dentine formation. The gilded sample of a labiolingually cut section of the 3rd mandibular molar (x 40). Dentine grew from left to right as indicated by a thick arrow. Six white arrows indicate the final points of lead acetate vital staining lines (2 mg. per kgm. intravenously injected at 4 days intervals, each at 8:30 a.m.). A, a part formed in 4 days before the operation; B, a part formed in 4 days after right unilateral adrenalectomy; C, a part formed in 4 days after 5~8 days of right unilateral adrenalectomy; E, a part formed in 4 days after 5~8 days of bilateral adrenalectomy. B is shorter, C is longer, and D and E are far shorter than the preoperative value A. Therefore, it is known that the speed of growth is temporarily decreased by right unilateral adrenalectomy, then it is increased, and again decreased remarkably after bilateral adrenalectomy. R. E., right adrenalectomy; L. E., additional left adrenalectomy.

Fig. 5. Effect of cortisone acetate upon periodic stripes on the dentine of a rabbit incisor. Microscopic figure of the labial side dentine of a rabbit incisor stained with hematoxylin (transverse section). Dentine grew from upper to lower as indicated by a large arrow. Five white arrows indicate the vital staining lines (2 mg. per kgm. each at 8:30 a.m.). a, a part formed in 4 days before the injection. M. cortisone acetate injection (dosage of 10 mg. per kgm. intramuscularly, immediately after the vital staining). b, a part formed in 2 days after the injection of cortisone acetate. On the following day of the injection, only an unstained stripe is formed by the injection. C, a part formed in 2 days after 3~4 days of the injection. d, a part formed in 4 days after 5~8 days of the injection. In c and d, only an intensively blue stained stripe is formed. This shows that the periodic stripes of dentine are affected by the injection of cortical hormone.
Moreover, there are many reports, e.g. by Sugimoto\textsuperscript{11}, Kisch\textsuperscript{6}, and others, which mentioned to the effect that the amounts of serum calcium and total protein have been decreased by adrenalectomy.

The results of the adrenalectomy which the author performed showed no substantial difference from previous reports regarding the influence on dentine calcification. In the present experiment, however, the author used the vital staining method with lead acetate, and took into account the factor of time marked on dentine section. In this way, the author could make the detailed changes unquestionably plain, which was impossible in the previous reports. For example, with reference to the lead lines by the vital staining which served as time index, the author could determine exactly to the minute at what time after the operation the variation of calcification occurred in dentine. At the same time, serum calcium and total protein were measured. So far there are no reports on the combined relation between dentine calcification, the amounts of serum calcium and total protein with reference to time. Really, new findings were rendered by the lead acetate vital staining.

Yonaga\textsuperscript{15} also observed the change of calcification on the rat dentine, caused by adrenalectomy, using the vital staining method. But compared with the Yonaga's, the author's results are more detailed thanks to the use of the rabbit dentine. The relation between the changes of serum calcium or total protein and dentine calcification caused by adrenalectomy is as follows: an unstained stripe corresponding to noncalcification is formed when the amount of serum calcium or total protein decreases, and a blue stained stripe corresponding to good calcification is formed when serum calcium increases. Therefore it is considered that the amounts of serum calcium and total protein decrease owing to adrenalectomy, which causes the deterioration of calcification. The formation of a succeeding blue stained stripe is assumed to be secondary result.

The influence of adrenalin on hard tissues is already made clear by Fuse\textsuperscript{3}, Kato\textsuperscript{5} and Okada and Mimura\textsuperscript{9}. But the author considers that the cortical hormone or adrenocorticotrophic hormone also plays an important part. There is no report on the influence of the cortical hormone or the adrenocorticotrophic hormone on the stripe figure of dentine. But the former is likely to influence on it (Fig. 5).

Now the author is making study on the detailed changes of calcification caused by cortical or adrenocorticotrophic hormone, which will be reported in the near future.

**SUMMARY**

The adrenalectomy of the rabbit is done one side after the other at eight days interval, and its effects on the dentine formation and growth, the amounts of serum calcium and total protein are observed with reference to the vital staining, and the following results are obtained.

The effect of adrenalectomy, observed both qualitatively and quantitatively, is that the calcification of dentine is retarded to some extent by unilateral ad-
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renalectomy, and remarkably by bilateral one, and the amounts of serum calcium and total protein decrease remarkably after adrenalectomy, and return to the normal level gradually.

As the cause of these effects, the cortical hormone is considered as important as the medullary hormone, which has been considered effective till now.

REFERENCES